

Amendments to the Specification

Please replace paragraphs [0036], [0043], [0044]  
with the following amended paragraphs:

[0036] **Figure 5** is a graphical representation of preparative anion exchange chromatography. Only a single chromatographically identified sphingosine kinase isoform is present in HUVEC. Preparative anion exchange chromatography with HiTrap-Q columns of human placenta, HUVEC and TNF $\alpha$  treated HUVEC extracts showing, in HUVEC, the presence of a single sphingosine kinase peak that increases in activity following treatment of cells with TNF $\alpha$ . Cells were harvested, lysed and the soluble extracts applied to the HiTrap-Q column in buffer A. Total sphingosine kinase activity in human placenta, HUVEC and TNF $\alpha$  treated HUVEC extracts were 51, 78 and 136 U/mg protein, respectively. Sphingosine kinase activity ~~(□)(●)~~ was eluted with a NaCl gradient of 0 to 1M (----).

[0043] **Figure 12** is a graphical representation of the physico-chemical properties of the native and recombinant sphingosine kinases. A, pH optima. The effect of pH on SK activity was determined by assaying the activity over the pH range of 4 to 11 in 50 mM buffers (sodium acetate, pH 4.0-5.0; Mes, pH 6.0-7.0; Hepes, pH 7.0-8.2; Tris/HCl, pH 8.2-10.0;

Caps, pH 10.0-11.0). *B*, pH stability. Data shown is the SK activity remaining after preincubation of the enzymes at various pH at 4°C for 5h. *C*, Temperature stability. Data shown is the SK activity remaining after preincubation of the enzymes at various temperatures (4 to 80°C) for 30 min at pH 7.4 (50mM Tris/HCl containing 10% glycerol, 0.5 M NaCl and 0.05% Triton X-100). *D*, Metal ion requirement. The various metal ions or EDTA were supplied in the assay mixture at a final concentration of 10 mM. In all cases the maximum activities of the native ( $\blacksquare(\bullet)$  and filled bars) and recombinant ( $\square\circ$  and open bars) sphingosine kinases were arbitrarily set at 100% and correspond to 2.65 kU and 7.43 kU, respectively. Data are means  $\pm$  S.D.

[0044] **Figure 13** is a graphical representation of the substrate specificity and kinetics of the native and recombinant sphingosine kinases. *A*, Substrate specificity of the native (filled bars) and recombinant (open bars) sphingosine kinases with sphingosine analogues and other lipids supplied at 100  $\mu$ M in 0.25% Triton X-100. The rates of phosphorylation of sphingosine by the native and recombinant Sks were arbitrarily set at 100% and correspond to 2.65 kU and 7.43 kU, respectively. Activity against other potential substrates were expressed relative to the activity against sphingosine. No phosphorylation was observed with DL-threo-

dihydrosphingosine, *N,N*-dimethylsphingosine, *N,N,N,-*  
*trimethylsphingosine, N-acetylsphingosine (C<sub>2</sub>-ceramide),*  
*diacylglycerol (1,2-dioctanoyl-sn-glycerol and 1,2-dioleoyl-*  
*sn-glycerol), and phosphatidylinositol.* *B, Substrate kinetics*  
*of the recombinant human sphingosine kinase with sphingosine*  
*(□)(●) and D-erythro-dihydrosphingosine (□)(○) as substrates.*  
*C, Kinetics if of inhibition of the recombinant human*  
*sphingosine kinase with N,N,N-trimethylsphingosine at 5 μM*  
*(□)(○) and 25 μM (□)(▼), and in the absence of N,N,N-*  
*trimethylsphingo-sine (□)(●).* Inset: Lineweaver-Burk plot.  
Data are means ± S.D.